

Selected formulations of *Bacillus cereus* strain SLBE3.1AP with different storage durations for control *Fusarium oxysporum* f. sp. *capsici* Chili Plants

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Abstract— The main disease in chili is fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* that can cause losses up to 100%. The aim of this study was to obtain a solid formula for the endophytic bacterium *Bacillus cereus* strain SLBE3.1AP with a carrier that can be stored longer and is effective for controlling *Fusarium oxysporum* f. sp. *capsici*. This research was an experimental study to see the ability of the solid formula of *Bacillus cereus* bacteria strain SLBE3.1AP in controlling *Fusarium oxysporum* f. sp. *capsici* using Completely Randomized Design consisting of 15 treatments and 3 replications. The treatments consisted of carrier material for the formula bagasse, rice straw, bran, fungicide and control. Each solid formula of *Bacillus cereus* strain SLBE3.1AP was introduced to chili seeds and seedlings. The results showed that the best formula for controlling *Fusarium oxysporum* f.sp. *capsici* and increasing the growth of chili plants was a solid formula of *B. cereus* strain SLBE3.1AP with 6 weeks storage of bagasse, 4 weeks of storage of rice straw, and 6 weeks of storage of rice bran.

Keywords— *Bacillus cereus*, chili, solid formula, *Fusariumoxysporum* f. sp. *capsici*, fusarium wilt.

I. INTRODUCTION

Red chili (*Capsicum annum* L) is one of the horticultural crop commodities that have high economic value, so it is widely cultivated by farmers (Saptana *et al.*, 2010). The higher demand for chili is sometimes not matched by the results of chili production. Chili productivity in Indonesia was relatively stable from 2014 to 2018, namely 8.35; 8.65; 8.47; 8.46; and 8.82 tons/ha (Central Bureau of Statistics, 2019). However, the productivity is still classified as low from the potential productivity of chili which can reach 12-15 tons/ha (Hadiyanti, 2016). One of the causes of the low productivity of chili is the attack of plant pathogens (Vivaldy *et al.*, 2017).

One of the main diseases of chili is Fusarium wilt caused by *Fusarium oxysporum* f. sp. *capsici* (Yanti *et al.*, 2020), Fusarium wilt disease results in losses and crop failure up to 50% if not controlled optimally (Rostini, 2011). *F. oxysporum* f. sp. *capsici* which is a pathogen of Fusarium wilt disease is a soil borne pathogen that is a soil inhabitant and can survive in extreme conditions with a chlamydiospore survival structure even in the absence of a

host. *F. oxysporum* f. sp. *capsici* infects plants through wounds on the roots and inhibits the flow of water in the xylem tissue causing the plants to wilt (Chehri *et al.* 2010).

Efforts to control *F. oxysporum* f. sp. *capsici* that have been recommended include mechanically removing diseased plants, resistant varieties, crop rotation (Sila and Sopilena, 2016), and synthetic fungicides active ingredient Mancozeb (Sari, 2020) which can have a negative impact on the environment. Based on this, an alternative control is needed, namely: by utilizing microorganisms as biological control agents (Natalia *et al.*, 2014). One of the biological agents that has been tested to control plant diseases and is widely used is endophytic bacteria (Sahu *et al.*, 2019). Endophytic bacteria are bacteria that live in plant tissues and do not cause disease or significant morphological changes in plants (Wang *et al.*, 2019).

One type of endophytic bacteria that has been widely used as a biological agent is *Bacillus* spp. because of its ability to sporulate and easily biodegradable by the environment. Biocontrol agents from *Bacillus* spp. including *B. pseudomycolides*, *B. mycolides*, *B.*

thuringiensis, and *B. cereus* (Yanti et al., 2019). The successful use of *Bacillus* spp. which can inhibit the growth of the *Fusarium oxysporum* (Diarta et al., 2016). The use of *Bacillus* spp. singly causes *Bacillus* spp. cannot last long and is less than optimal both as a biocontrol agent and as a bioactivator, this is because bacteria need nutrients so that bacterial formulations need to be made (Oktrisna et al., 2017; Yanti et al., 2017).

The aim of the study was to obtain a solid formula of *B. cereus* strain SLBE3.1AP which was effective in controlling *Fusarium oxysporum* f. sp. *capsici* on chili plants.

II. METHODS OF RESEARCH

The research was carried out on March-September 2021 at the Microbiology and Phytopathology Laboratory, Department of Plant Diseases Pests and Experimental Gardens, Faculty of Agriculture, Andalas University, Padang. Study is experimental using Completely Randomized Design (CRD) with 15 treatments and 3 replications. The treatment consisted of formula *B. cereus* strain SLBE3.1AP with various carrier materials from organic waste, namely: bagasse (AT), bran (D) and rice straw (JP) storage 0, 2, 4, and 6 weeks with a ratio of 1 :1 (v/v), fungicide treatment with the active ingredient Mancozeb, treatment without formulation and without inoculation of *F. oxysporum* f. sp. *capsici* (positive control), treatment without formulation and inoculation with *F. oxysporum* f. sp. *capsici* (negative control).

Propagation of *B. cereus* strain SLBE3.1AP

Propagation of *B. cereus* strain SLBE3.1AP was carried out in liquid culture by means of pure cultures of *B. cereus* strain SLBE3.1AP aged 2x24 hours were taken, put into 25 ml of NB medium in a culture bottle (volume 50 ml) and incubated on a rotary shaker for 24 hours. Next, 1 ml of preculture results were transferred to 49 ml of sterile coconut water in a culture bottle (100 ml volume) for mainculture and incubated in the same way for 2x24 hours at 150 rpm. Population density was determined by comparing the turbidity of the bacterial suspension with a McFarland scale 8 solution (population density estimated at 10^8 cells/ml) (Klement et al., 1990).

Preparation of solid formula carrier *B. cereus* strain SLBE3.1AP

The soft part of the bagasse is taken and cut into small pieces and then blended, the tofu pulp is put into aluminum foil and then baked, the rice straw is cut into small pieces then blended and the bran is filtered to get a smooth part. 9.5 g of each carrier was taken and put into a 100 ml Schott bottle and added 0.5 g of sucrose, then

sterilized using an autoclave at 1 atm pressure at 121°C for 15 minutes. The formula is cooled and 5 ml of suspension is added *B. cereus* strain SLBE3.1AP from mainculture 10^8 cells/ml. Each formula was stored at room temperature and incubated for 0, 2, 4, and 6 weeks (Yanti et al., 2017).

Pathogenicity Test

Foc inoculated by injuring the roots of the chili plants with scissors, then the 10 g *Foc* rice substrate was immersed into the soil 3 cm around the roots of the chili plants that had been injured. If the plant shows symptoms of wilting, the inoculum is classified as a pathogen (Chamzurni et al., 2010).

Propagation *Foc*

Propagation of *Foc* using rice media, as much as 2.5 kg of rice divided by 10 g for each treatment. The rice is washed and then the rice is dried, then put into a plastic 10x20 cm and sterilized in an autoclave at a temperature of 121°C for 30 minutes. After the cold rice, the *Foc* cultures were cut 1x1 cm to be inoculated into the rice substrate and incubated for 21 days.

Introduction of formula *B. cereus* strain SLBE3.1AP

The introduction of the *B. cereus* formula SLBE3.1AP strain was carried out 2 times, namely at seeding and planting for 15 minutes.

Inoculation *Foc*

Before planting, the planting medium was inoculated with *Foc* 1 g. The *Foc* rice substrate was transferred to a test tube containing 10 ml of sterile distilled water, then homogenized with a vortex, the suspension was taken with a dropper and the number of conidia was counted using a haemocytometer under a microscope with a magnification of 40x10. The population used for inoculation into chili plants was 10^6 conidia/ml. *Foc* was inoculated by immersing 9 g of *Foc* rice substrate into the soil to a depth of approximately 3 cm to maintain soil moisture, the inoculated planting medium was covered with transparent plastic for 3 days, so that *F. oxysporum* f. sp. *capsici* grows well (Chamzurni et al., 2010).

Observation

Observations were made on disease development, seedling growth and chili plant growth. The data were analyzed by means of variance, if significantly different then continued with Least Significance Differences (LSD) at the 5% level.

III. RESULTS AND DISCUSSION

The introduction of a solid formula of *B. cereus* strain SLBE3.1AP with different storage times in chili plants, the results showed that all introduced formulas were able to suppress the development of *Fusarium* wilt disease. All formulas of *B. cereus* strain SLBE3.1AP were stable in suppressing incubation period, disease incidence and severity of *Fusarium* wilt disease in chili plants. This is presumably due to the type of formula, the nutritional content and the shelf life of the formula, the formula comes from endophytic bacteria which has many enzymes and carriers contain nutrients needed by bacteria to thrive. The best formula has a shelf life of 6 weeks during which time *B. cereus* can produce several resistance compounds. This is in accordance with the opinion of Taghavi *et al.*, (2005) in Yuniawati *et al.*, (2019) endophytic bacteria are able to produce enzymes, salicylic acid, ethylene and secondary metabolite compounds that play a role in inducing plant resistance. According to Hallman *et al.*, (2009) in Munif (2003) before pathogens attack plants, endophytic bacteria that have been associated with plants can act as biological control agents, this is in accordance with the method in this study, namely the introduction of solid formula *B. cereus* strain SLBE3.1AP before sowing and planting chili seeds. It is hoped that the antagonist bacteria will be able to suppress the growth and development of the fungus *F. oxysporum* which in turn can reduce the attack rate. The results of this study are also in accordance with the research of Yanti *et al.* (2017), which states that there are 5 isolates of indigenous endophytic bacteria are one of them *B. cereus* strain SLBE3.1AP capable reduce the incidence of *Fusarium* wilt up to 100%.

The solid formula of *B. cereus* strain SLBE3.1AP introduced into chili seeds was able to increase chili growth in the seedling phase. The formula was able to increase seedling field emergence, seedling height, number of seedling leaves, wet weight and dry weight of seedlings compared to control (without treatment). From the research results, the stable formula in increasing seedling growth with 100% effectiveness was the rice straw formula which was stored for 4 weeks and the bagasse formula which was stored for 6 weeks. This is presumably because the best formula is a combination of carriers, which means that the more carriers there are, the more nutrients are contained in the formula, thereby increasing root fertility and plant growth.

Research result This is in accordance with the research of Resti, *et al.* (2018) which reported that endophytic bacteria can increase seedling growth and emergence of chili seedlings by 95% compared to controls. According to Lisnawita *et al.* (2016), the increase in plant growth, both plant height and number of leaves, was positively correlated with the contribution of hormones

produced by endophytic bacterial isolates. This is in line with the results of research by Marum *et al.* (2012), who reported that the application of bagasse can increase leaf area, dry weight and fresh weight of plants and provide effective yield growth of radish plants. This is because the organic content of bagasse can reach 50% and has great potential as a source of organic matter that is useful for soil fertility (Ayu, 2018). Furthermore, Aldi *et al.* (2016) reported that the PGPR formulation in chili can accelerate the emergence of chili flowers at the age of 54 DAP with an average flower appearance of 84% of the total experimental plants and increase the number and weight of chilies.

IV. CONCLUSION

The best formula for controlling *Fusarium oxysporum* f.sp. *capsici* and increasing the growth of chili plants was a solid formula of *B. cereus* strain SLBE3.1AP with 6 weeks storage of bagasse, 4 weeks of storage of rice straw, and 6 weeks of storage of rice bran.

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FIGURES AND TABLES

Table 1. The development of *Fusarium* wilt disease in chili plants introduced by each treatment

Treatment		Disease Progress						
Carrier Material	Storage Time	Incubation Period	Effectiveness (%)	Disease Incidence (%)	Effectiveness (%)	Disease Severity (%)	Effectiveness (%)	Attack Criteria
Bagasse	0	21.00 cde	117.16	100.00 a	0.00	41.67 ab	16.53	Heavy
Bagasse	2	29.67 abc	206.82	33.33 ab	66.67	16.00 bc	63.90	Light
Bagasse	4	30.67 ab	217.16	33.33 ab	66.67	14.00 bc	68.41	Light
Bagasse	6	34.00 a *	251.60	0.00 b*	100.00	0.00 c*	100.00	Healthy
Rice straw	0	19.00 de	96.48	100.00 a	0.00	37.67 ab	15.02	Heavy
Rice straw	2	27.33 abcd	182.62	66.67 ab	33.33	24.67 abc	44.34	Currently
Rice straw	4	29.67 abc	206.82	33.33 ab	66.67	15.00 bc	66.16	Light
Rice straw	6	30.67 ab	217.16	33.33 ab	66.67	16.33 bc	63.16	Light
Bran	0	18.33 ef	89.55	100.00 a	0.00	39.33 ab	15.02	Heavy
Bran	2	30.67 ab	217.16	33.33 ab	66.67	16.33 bc	63.16	Light
Bran	4	30.67 ab	217.16	33.33 ab	66.67	15.67 bc	64.65	Light
Bran	6	30.67 ab	217.16	33.33 ab	66.67	11.67 bc	73.67	Light
Mancozeb	-	24.33 bcde	151.60	66.67 ab	33.33	30.33 abc	42.09	Currently
+ control	-	34.00 a *	251.60	0.00 b*	100.00	0.00 c*	100.00	Healthy
Control -	-	9,667 f	0.00	100.00 a	0.00	54.00 a	0.00	Very heavy

*plants did not show symptoms until the end of observation (34 DAI)

*Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.

Table 2. Growth of chili seedlings introduced for each treatment

Treatment		Seed growth										
Carrier Material	Storage Time	Power appears field	Effectiveness (%)	Seedling height (cm)	Effectiveness (%)	Number of leaves (strands)	Effectiveness (%)	Effectiveness (%)	Wet weight (gam)	Effectiveness (%)	Dry weight (gam)	Effectiveness (%)
Bagasse	0	96.00	27.78	11.00 ij	26.87	5.66 bcde	62.00	26.08	0.12 jkl	33.33	0.02 ghij	00.00
Bagasse	2	96.00	27.78	11.50 gh	32.64	5.33 de	52.28	78.26	0.23 cd	155.55	0.05bcde	150.00
Bagasse	4	96.00	27.78	10.33 k	23.06	5.99 ef	42.85	78.26	0.14 ghijk	55.55	0.04 efgh	100.00
Bagasse	6	100.00	33.33	12.33 de	42.21	6.33 ab	80.85	147.82	0.26 bc	188.88	0.06 abc	200.00
Rice straw	0	96.00	27.78	11.67 fg	34.60	5.33 de	52.28	43.47	0.12 cl	33.33	0.03 fghi	50.00
Rice straw	2	96.00	27.78	11.00 ij	26.87	6.00 bcd	71.42	113.04	0.19def	111.11	0.04 def	100.00

Rice straw	4	100.00	33.33	12.83 bc	47.98	5.66 bcde	62.00	113.04	0.22 cd	144.44	0.05 cde	150.00
Rice straw	6	100.00	33.33	12.00 ef	38.40	5.66 bcde	62.00	104.34	0.15 ghijk	66.67	0.05 cde	150.00
Bran	0	92.00	22.22	11.33 ghi	30.68	5.33 de	52.28	39.13	0.08 l	- 4.44	0.01 j	- 50.00
Bran	2	100.00	33.33	12.33 de	42.21	5.33 de	52.28	69.56	0.15 ghijk	66.67	0.02 ghij	00.00
Bran	4	96.00	27.78	10.66 jk	19.14	5.66 bcde	62.00	95.65	0.13 hijk	44.44	0.07 ab	250.00
Bran	6	96.00	27.78	12.50 cd	44.17	6.00 bcd	71.42	56.52	0.20 de	122.22	0.04 efgh	100.00
Mancozeb	-	92.00	22.22	11.16 hi	28.83	5.16 ef	47.71	21.73	0.17 efgh	88.89	0.02 hij	00.00
Control	-	75.00	00.00	8.67 l	00.00	3.50 g	00.00	00.00	0.09 l	00.00	0.02 ij	00.00

*Numbers followed by the same lowercase letter in the same column are not significantly different according to LSD at the 5% level.